The Development of the Temnocephaleæ.
Part I.

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With Plates 23—25.

I. Introduction.

Though several additions of importance[1] have been made to the literature concerned with Temnocephala and its allies since 1893, when I published an account of the group under the title "A Monograph of the Temnocephaleæ" (**8**), no attempt has been made hitherto to deal with the embryology of any of its members. In view of the interest which attaches to them on account of their isolated character and problematical relationships with other sections of Platodes, it is very desirable that something should be done towards filling this hiatus in our knowledge.

What follows is by no means a complete or exhaustive account of this subject. It is concerned mainly with the developmental history of a single form—viz. Temnocephala fasciata—one of the most widely distributed of the Australian representatives of the group: and in this

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[1] The most extensive and important of these is that of Wacke (**16**), published in 1900. When writing this the author had, apparently, not had the opportunity of consulting my "Monograph," and all his references, critical and otherwise, concern themselves with a short paper which I published in 1888 in the 'Quarterly Journal of Microscopical Science.' Hence there is a good deal that goes somewhat wide of the mark.

history there are a number of important points that have not yet been determined—notably the processes of maturation and fertilisation, and the early segmentation phases. Since, however, the portions of the development which I have succeeded in tracing reveal certain phases that are quite unique in character, it appears to me expedient to publish these observations as a first contribution to our knowledge of the subject.

In addition to Temnocephala fasciata, T. minor, T. dendyi, and T. quadricornis, as well as Craspedella spenceri, have been made the subjects of study; but in none of these were the methods adopted with the eggs sufficiently satisfactory in their results to enable me to do more at present than state that the general course of the development is the same in all these forms. Of the somewhat specialised New Zealand species—T. noviæ-zealandiæ—I have procured ample material, which is now in course of preparation.

What has impressed me most strongly in connection with this investigation has been the complete absence in the development of any definite evidence of relationships with the groups looked upon as the most nearly allied. Until comparatively recently little that could be accepted as well authenticated had been published on the development either of the Rhabdocœles or of the Heterocotylean Trematodes. In the latter group as yet little has been done since the publication of Zeller's (**17**) account of the development of Polystomum in 1876. But Bresslau's valuable work (**3**) has furnished us with a much-needed body of information on certain Rhabdocœles, and this has enabled me to effect a comparison with Temnocephala, with the result that the differences appear to be more numerous and more radical than the resemblances. So far as can be ascertained in the present state of our knowledge, a similar result follows from a comparison with the Heterocotylean Trematodes. The upshot seems to be that the study of the development, so far as it has been carried at present, does not in any way tend to bridge over, but rather to widen, the gap between the Temnocephaleæ and neighbouring groups.

II. Methods.

Though the eggs are abundant and readily procurable, the study of the development of Temnocephala presents considerable technical difficulties, owing to the intractable character of the material. The egg-shell is tough and relatively thick, and not readily permeable by reagents. When it is broken through, the contents, in the fresh condition, burst out, and become completely disorganised. When fixed and hardened in the ordinary way the yolk becomes extremely hard and brittle. Many methods were experimented with before the following course of procedure, which has proved sufficiently satisfactory, was finally arrived at.

The eggs are fixed with sublimate alcohol followed by iodized alcohol and 90 per cent. alcohol. After hardening they are treated with a solution of hypochlorite of soda. If the eggs are transferred directly from the strong alcohol to the hypochlorite solution, the shell of most of them splits longitudinally, and, before the desired effect in softening and removing the egg shell has been attained, the contents, completely exposed, are disintegrated. This effect is avoided by making the transference gradually through downwardly graded alcohols to the watery solution.

A weak solution of the hypochlorite soon dissolves the cement that attaches the eggs to one another, and begins to act on the substance of the egg-shell itself. When the action is judged to have proceeded far enough, the eggs are washed in distilled water, and then dehydrated with alcohol. Double embedding is essential. From absolute alcohol the eggs are transferred to a mixture of equal parts of absolute alcohol and anhydrous ether, in which they remain for twenty-four hours. They then remain for a like period in ½ per cent. solution of photoxylin (or celloidin) in equal parts of absolute alcohol and ether, followed by a 2½ per cent. solution of the same. The celloidin blocks, hardened in chloroform, are then finally embedded in the hardest paraffin in the usual way. The staining agents employed almost exclusively were Ehrlich's hæmatoxylin, or Mayer's hæmocalcium, followed by eosin.

III. Formation of the Egg.

The ovary (germarium) in Temnocephala fasciata, and in all the Australasian species of the genus (Pl. 23, fig. 1), is a solid ellipsoidal mass of ova enclosed in a thin capsule of muscular fibres. At the right extremity, which is the one situated nearest to the oviduct, is the largest ovum, which is more rounded in form than the rest. The remainder decrease gradually in size towards the left, the largest of them extending across the entire width of the ovary. At the left end is a mass of smaller ova, which show evidence of slow multiplication by mitotic division. The full-grown ovum, at the right-hand end of the ovary, is about 0·11 mm. in long diameter. Its protoplasm is densely loaded with very fine granules, and contains, in addition, a number of much larger rounded masses of very definite spherical form. The nucleus is large, about one third of the diameter of the ovum itself, with spherical nucleolus, and a fine, open, achromatin network.

The ripe ovum becomes detached from the others, and passes into the oviduct, which opens through the capsule of the ovary. It must then pass along the oviduct to the ootype, where it becomes surrounded by a mass of yolk-cells and the whole then becomes enclosed in a chitinous shell, the substance of which is secreted by the shell glands.

Considerable differences in detail distinguish the various species of Temnocephala as regards not only the male parts of the reproductive apparatus, but also the female. But in all the species which I have had the opportunity of examining, the essential features of the parts concerned in the formation of the egg closely correspond. The two main vitelline ducts, right and left, open together into the oviduct near the ovary. Close to this the oviduct gives off a short branch leading to a large sac, with a syncytial epithelium, lying near the middle line in front of the rest of the female apparatus. This has been very usually called the receptaculum seminis—a name for which, in view of the fact that its contents consist very largely of yolk matter, I proposed (8) to substitute that of receptaculum vitelli. I was then of opinion that it serves as a receptacle in which yolk accumulates until enough has been collected for completing an egg, when it is discharged into the ootype. A more thorough examination of the subject has shown me, however, that, at least as regards the Australasian forms, both names are inappropriate. The sac usually contains spermatozoa it is true, but they never form a large proportion of its contents; and they are spermatozoa which have lost their activity, and move, when they move at all, with comparative sluggishness. It also contains yolk matter—the great bulk of the contents, in fact, consisting of that material, but it is yolk matter which has undergone degeneration; the cells have broken up, and the nuclei have, for the most part, disappeared. Moreover, mingled with the motionless or sluggish spermatozoa and the broken-down yolk-cells are shreds and strands of a substance which corresponds exactly in appearance and behaviour to staining agents with the secretion of the shell-glands. The conclusion to be arrived at from these facts is clear enough; the so-called receptaculum seminis, or receptaculum vitelli, is in reality a receptacle for surplus spermatozoa and surplus vitelline matter as well as shell-gland secretion.

The question may suggest itself—What necessity is there for such a receptacle? Why should the surplus matter not be passed directly out through the female duct? To this the answer obviously is that very frequently—whenever, in fact, an egg is in course of formation in the ootype or is lodged in the distended atrium—the way to the exterior is blocked, and in order that the formation of the egg may proceed without interference, the yolk-cells which are being discharged into the oviduct, and the shell-gland secretion which collects after the shell has become formed, as well as the surplus spermatozoa, have to be disposed of.

That this is the function discharged by the receptaculum in Temnocephala there remains, to my mind, not the slightest doubt. The function of a true receptaculum seminis in those animals is discharged by the anterior part of the oviduct, in which a mass of actively moving spermatozoa is usually to be found. T. comes has an exceptional arrangement of the parts; in that species the cavity of the receptaculum is incompletely divided into two unequal parts by a partition which is pierced in the middle by a wide aperture. In all the specimens I have examined, while the distal larger part of the cavity, the part furthest from the oviduct, is filled with the usual mixture of effete genital products, the smaller part is occupied by a mass of normal spermatozoa.

Whether the relatively large sac known as receptaculum seminis in other Platodes may perform in some cases the same function as that above ascribed to it in Temnocephala is a question worthy of further investigation. Where, as in many Distomids, a Laurer's canal is present that canal seems to be the natural outlet for the unused and effete materials; when as in Distomum nodulosum, D. globiporum, D. isoporum, and others (Looss, **11**), a receptaculum is present as well, it may act as a true receptaculum seminis. When a Laurer's canal is absent the receptaculum in some forms—D. variegatum (Looss, l.c.)—contains yolk-cells as well as spermatozoa. Looss expresses the opinion that, in general, the spermatozoa contained in the receptaculum seminis of Distomids are in process of dissolution, and no longer capable of effecting fertilisation. If this be true of the Distomids as well as Temnocephala, it is at least possible that the same may prove to be true of the other groups—Polyclads, Heterocotylean Trematodes.

IV. The completed Egg.

The eggs of all the species of Temnocephala, on being discharged, are fixed by a chitinoid cement to some part of the outer surface of the body of their hosts. In some of the species attachment is effected through the intermediation of a longer or shorter stalk situated at one end. Such stalked eggs occur in T. chilensis according to Monticelli (**14**), Plate (**15**), and Wacke (**16**). Similar stalks occur also in T. novæ-zealandiæ and in T. minor. The cementing material usually extends between the stalks of neighbouring eggs, thus uniting them into groups as observed by Monticelli and by Wacke. In such stalked eggs an operculum may be formed, when the young animal is ready to become free, by the formation of a circular split in the egg-shell near the distal end. In both T. novæ-zealandiæ and T. minor there is a short filament attached near the middle of the distal end. T. fasciata, T. comes, T. dendyi, T. semperi, and T. quadricornis have more or less elliptical eggs which have no stalk, but are cemented down by one side, a number being, in most cases, united together by means of the cementing material. Of these the eggs of T. fasciata, T. comes, T. quadricornis, T. semperi are provided with filaments, those of T. dendyi are devoid of them.

The size of the egg is, in general, in relation with the size of the adult. The larger species—T. fasciata, T. quadricornis, and T. novæ-zealandiæ—have comparatively large eggs, about 5 mm. in length. The minute Craspedella spenceri, at the other extreme, has oval sessile eggs, without filaments, which are only 0·2 mm. in diameter.

Temnocephala, like its allies the Rhabdocœles and the Heterocotylea, has no larval stage; the young animal, when it escapes from the egg, differing from the adult only in its small size, and in the reproductive apparatus not having attained to complete development.

The older eggs are to be recognised by the eyes, which are visible through the shell. These may be placed in such a way as to show that the young Temnocephala is lying with its long axis parallel with that of the egg, but in a large number of cases the position is a transverse one, and occasionally an intermediate condition occurs. This variation in the direction of the long axis is not due to movement of the larva in its later stages; the direction varies from the outset, and regulating the direction of sectioning, except in advanced embryos, is little more than mere guesswork.

After the ovum has become fertilised, and the egg completed by the addition of the mass of yolk-cells and the enclosing shell, it appears to pass without much delay into the genital atrium, which serves the purpose of a uterus. Here it may remain some little time before passing out through the genital aperture; but the stage of development which has been attained when the deposition takes place varies. In most cases an egg from the ootype or atrium contains an ovum in which the process of segmentation has not yet begun; and unsegmented ova are occasionally found among those attached to the surface of the crayfish; but occasionally segmentation is found to be well advanced in a uterine egg. I have never found more than one egg in the uterus.

When the egg is fully formed, the greater part of its mass consists of yolk-cells. These are polyhedral cells of an average diameter of about ·050 mm., with granular contents. Each has a nucleus ·015 mm. in long diameter, of oval or elliptical shape, with a single spherical nucleolus with a diameter of about ·005 mm. The yolk-cells are at first quite distinct from one another (Pl. 23, fig. 2); but, as development proceeds, a gradual coalescence takes place, beginning at the periphery, and eventually (Pl. 24, fig. 7) the entire mass completely fuses to form a syncytium, in which all trace of cell outlines has become completely lost. When the formation of the syncytium has begun, the nuclei of the more superficial cells pass outwards and come to lie close to the surface, so that they present the appearance, to some extent, of the nuclei of a superficial epithelium.

The ovum (Pl. 23, fig. 2) is embedded in the yolk-cells, usually towards the middle, sometimes towards one end of the egg. It is a polyhedral cell, ·08 mm. in diameter, with a finely granular protoplasm that stains much more deeply than the substance of the yolk-cells. In all the specimens I have had the opportunity of examining the nucleus has undergone modification, and is represented by a cluster of clear vesicles, each enclosing a rounded particle with the staining affinities of chromatin.[1]

In two cases I found an egg containing two cells. The nuclei of both had undergone the modification just described. One of the cells was very much smaller than the other, and on that account it seems to be more probable that we have here to do with the first stage of segmentation rather than with an egg in which two ova had become enclosed.

V. Early Development.

The process of segmentation results in the formation of a blastoderm of irregular shape, which comes to be drawn out in the direction of the long axis of the future worm—a direction, as already explained, usually parallel with the long axis of the egg, but not invariably so. No germinal layers are recognisable: but from a very early stage (Pl. 23, fig. 3) the blastoderm is found to consist of three sets of cells, which differ from one another in a very marked manner in their size and in the character of their nuclei. The cells of one set are 0·03 mm. in diameter, have nuclei about 0·015 mm. in diameter, each containing a large rounded nucleolus. Those of the second set are 0·125 mm. in diameter, have smaller nuclei, 0·0075 mm. in diameter, usually without nucleoli, but with a rather close network. The cells of the third or smallest set are 0·0075 mm. in diameter, with correspondingly small nuclei. No definite arrangement of these cells is recognisable until a few of the larger cells become grouped together (Pl. 24, fig. 5) in such a way as to bound a small rounded cavity. This elongates and widens (Pl. 24, fig. 6), the bounding cells meantime increasing in number. Eventually (Pl. 24, figs. 7, 8, 9, and 10) the space dilates very greatly, the cells which form its walls becoming correspondingly extended, and uniting together to form a comparatively thin membrane with flattened nuclei. As it enlarges, this space becomes approximated towards the surface of the egg, coming to be separated from the shell only by a thin layer of yolk. In apposition with the deeper side of the space lies the main mass of the blastoderm, which is rapidly increasing in extent, the increase being mainly due to the multiplication of the middle-sized cells.

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[1] Somewhat similar appearances were observed by Zeller (**17**) in Polystomum.

The space above referred to does not correspond, so far as I have been able to ascertain, to anything that has been found to occur in any other group of animals. Since it plays an important part in development, it is necessary to have a name for it, and I propose the term endocœle as one not involving any dubious homologies.[1]

The rudiment of the brain (Pl. 24, figs. 8, 9, and 10, *br.*) makes its appearance as the endocœle approaches its maximum size. It appears first as a bilobed, dense aggregation of cells on the deeper dorsal side of the endocœle, a little distance from the lining membrane. In the middle of this appears a transversely elongated space filled with finely fibrillated matter—"Punktsubstanz." Nerve-fibres (or nerve-tubes) are only developed in the latest embryonal stages. From the central mass a pair of processes—the foundations of the peripheral nervous system—are given off laterally.

About the same time as the beginnings of the nervous system, appears the first rudiment of the excretory system of vessels. This takes the form of several specially modified large cells, on either side of the rudiment of the brain, and somewhat behind it. These cells are situated immediately below the thin epithelial lining of the endocœle cavity. One cell becomes considerably enlarged, and a narrow sinuous channel becomes formed in its substance. This channel becomes continued through a second and a third cell placed in close apposition with the first. As subsequent stages show, these constitute the rudiments of the terminal contractile sacs and the beginnings of the main vessels of the excrctory system.

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[1] The same cavity with the same relations occurs in Craspedella as well as Temnocephala.

The pharynx is formed from a number of cells which become arranged after the manner of an epithelium immediately beneath (i. e. outside of) the thin syncytial epithelium of the endocœle on the dorsal side. The time of appearance of this layer varies somewhat. Usnally it is not seen until both the brain and excretory rudiments have become well established. The part of the wall of the cavity on which it is situated becomes somewhat rounded off, though still remaining in wide communication with the rest. Posteriorly a short prolongation without cellular lining extends for a short distance backwards into the mass of yolk; this represents the lumen of the intestine (see Pl. 24, fig. 11).

The cavity of the endocœle as a whole decreases much in size. The thin layer of yolk by which it is separated from the exterior becomes still more attenuated, but still remains as a definite septum (Pls. 24 and 25, figs. 11, 12, 13, *s.*), cutting off the whole internal cavity from the exterior.

When the brain and the excretory sacs are first formed, the blastoderm does not extend anteriorly or posteriorly beyond the limits of the endocœle. But a little later it begins to grow backwards to form the foundations of the posterior parts of the embryo. This backward extension (Pl. 25, fig. 14) is made up, like the main body of the blastoderm, of cells of three sizes, small, intermediate, and large; and these are arranged in groups with a marked bilateral symmetry. The large cells are formed by a proliferation of the membrane lining the endocœle. Outlying small cells are to be found here and there embedded in the yolk, at a little distance from the main body.

In the next stage observed this posterior elongation of the blastoderm has reached the ventral surface, in what is destined to be the genital region, and has become almost completely separated off from the anterior part, in which the further development of the brain, the pharynx, and the excretory system is going on. There thus come to be two distinct foci of development, an anterior and a posterior. From both superficial cells must be separated off to form the epidermis, since this layer is to be recognised as a distinct, though very thin layer, with widely separated nuclei, at the stages when the tentacles and sucker first begin to be formed. This layer is early completed on the ventral surface, but on the dorsal surface it does not make its appearance till a considerably later stage.

The rudiments of the tentacles (Pl. 24, fig. 11) make their appearance at a stage when certain changes in the cavity have led to the first differentiation of the pharynx, and when the rudiments of the eyes have first become distinguishable. They appear as processes which grow at first straight forwards from the anterior extremity of the body; but as they elongate, they become bent (Pl. 25, figs. 12, 13), usually towards the ventral, but in many cases towards the dorsal, side. In the former case the cephalic portion of the body (Pl. 25, fig. 13) becomes strongly flexed ventrally at the same time. When this takes place the part of the surface covered by the reflexed tentacles develops a system of minute tooth-like epidermal papillæ, which are apparently of firm consistency.

The rudiment of the sucker makes its appearance about the same time as those of the tentacles. Epidermal papillæ, similar to those underneath the tentacles, are formed under the sucker.

We have left the endocœle as an extensive rounded space, occupying nearly a third of the length of the egg, with a floor and a roof. As the cavity reaches its greatest dimensions the layer of yolk, which separates it on the ventral side from the exterior, becomes greatly reduced in thickness. At the same time, subsequently to the formation of the embryonic brain and excretory sacs, a portion of its wall just behind the brain undergoes modification, a number of large cells becoming arranged in an epithelium-like manner beneath the thin lining membrane. The cavity then becomes much reduced in size, and the part with the large cells becomes rounded off to form the pharynx. Ventrally it continues to be separated from the exterior by the original roof of the cavity, which becomes reduced to an extremely thin membrane. Posteriorly a further remnant of the original cavity is represented by a very short passage which ends blindly in the yolk. The pharyngeal sac and mouth become formed by growth of the integumentary and muscular layers around the thin membrane that represents the original roof of the cavity; but the membrane remains as a distinct, though very delicate, partition between the buccal cavity and the pharynx as long as the young animal remains within the egg.

The intestine remains without lumen in the most advanced stage observed within the egg, a stage in which all the other parts, even the male reproductive apparatus, have reached an advanced stage of development. It would, perhaps, be more correct to say that the young animal has no intestine at this stage, the site of the future intestine being occupied by a solid mass of yolk still containing remains of the original nuclei of the yolk-cells.

VI. Development of the Excretory System.

The excretory system of the adult Temnocephala, though constructed on the same type as that of the Platodes in general, possesses certain features which, so far as our present knowledge extends, are peculiarly its own. The presence of contractile terminal sacs through which the system communicates with the exterior is not peculiar to this group, similar structures occurring in some of the Heterocotylean Trematodes (Braun, **2**; Goto, **4**). But the nature of these sacs and their relations to the system of vessels in Temnocephala are quite unlike anything that is known to occur in other forms.

The most essential features of this system were described by me in 1893 (**8**). But since some of the most important of these points have been overlooked by recent writers,[1] or their significance not recognised, it seems desirable to give a brief résumé of the facts.

Each of the two dorsally situated excretory apertures leads into a thick-walled contractile terminal vesicle (Pl. 25, fig. 18), which is of pyriform shape, bent on itself towards its apex. The contractions of the walls of the vesicle are effected by the agency of an enclosing layer of muscular fibres, and the external aperture is surrounded by a muscular sphincter. Between the muscular layer and the proper wall of the vesicle is a layer of loose parenchyma, which doubtless facilitates freedom of movement. The vesicle itself consists of two large cells fused together and hollowed out to form the lumen. The greater part of the wall of the vesicle is formed by one of these two cells; the narrower apical part, and the beginning of the main duct which is given off from it by the other. The relative position of the two cells is indicated mainly by the position of their two nuclei, but the texture of the protoplasmic substance of the two cells differs somewhat in character, and that of the smaller is much more susceptible to the action of staining agents.

The inner surface of the vesicle is quite smooth and uniform. Where an occasional exception to this appears to occur, and the usually sharp internal outline appears blurred, and the lumen is occupied by fine filamentous matter that might be mistaken for cilia, this occurs in such an irregular way that I have little doubt that this appearance is due to a rupture or other alteration that has occurred during the fixing process. In no case, either in Temnocephala novæ-zealandiæ or any other form, is there an epithelium with scattered nuclei, as supposed by Wacke. In some preparations the internal contour appears double, as if the cavity of the vesicle possessed an excessively thin cuticular lining, but this is always very indefinite, and in many cases is not to be detected. The only nuclei in the entire organ are the two already referred to as the nuclei of the two constituent cells.

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[1] Plate (**15**) and Wacke (**16**) for example. The former states, "An den Nephridien der Temnocephaliden ist bis jetzt vergeblich nach den für die Platyhelminthen so characteristischen Flimmerzellen gesucht worden." Yet I had described the system with its flame-cells in a paper in the 'Zoologischen Anzeiger' two years before (1892).

Arising from the main excretory trunk at a little distance from the vesicle is a special branch of considerable size—the vesicular vessel as it may conveniently be termed. This runs inwards, and enters the wall of the vesicle on its inner side. Here it breaks up (Pl. 25, fig. 19) into a number of branches, which ramify throughout the protoplasmic substance of the wall of the sac in all directions. In the course of the system of fine intracellular capillaries which is thus formed occur numerous ciliary flames of small size, but in other respects similar to the ciliary flames in the flame-cells of other Platodes. I have counted as many as fifty of these ciliary flames in movement at one time in the case of T. novæ-zealandiæ, and probably many more than that number are actually present.

In sections of the terminal vesicle in all the Australasian species the ramifications of the vesicular vessel are very conspicuous, pervading the protoplasmic wall in all directions. But the ciliary flames are not to be made out with any certainty save in the living animal.

In most cases the inner part of the protoplasmic wall in sections appears regularly divided by fine parallel vertical lines, and one might be tempted to suppose that these represent a system of vertical canals forming outlets from the system of vesicular capillaries into the lumen of the vesicle.

From the unbroken appearance of the limiting line of the surface on which the vertical lines terminate, I incline to the opinion that no such communications exist.

The details of the arrangement of the vessels differ in the different species. The main trunk soon bifurcates to form anterior and posterior main vessels, which give off numerous branches to all parts of the body. A large vessel runs along the axis of each of the tentacles.

Given off from the larger vessels in the body is a system of fine, thin-walled capillaries, which are most abundant near the dorsal surface, where they form an extensive plexus.

A limited number of ciliary flames are to be detected in the living animal distributed throughout various parts of the body and the tentacles. The relation of these to the vessels of the excretory system still remains undetermined. In no case was a nucleus observed in close relation with the ciliary flame.

The walls of the larger vessels consist simply of a fine-grained, structureless protoplasmic material. Here and there, usually at long intervals, are the nuclei of the elongated cells of which the walls are composed. These are comparatively few in number even in the larger species. Their presence and their relations to the vessels are best observed in longitudinal sections—most readily in the tentacles, in which such appearances as those represented in figs. 16, 17, 18 of pl. x of my "Monograph" are readily recognisable.

Some of the excretory vessels end in certain specially modified large excretory cells. The branch in question, sometimes fairly thick-walled, sometimes very delicate, enters the cell and breaks up into a richly ramifying and anastomosing system of minute capillaries within its substance.

The first trace of the system of excretory vessels makes its appearance at a very early stage in the history of the embryo. In a blastoderm in which the endocœle has become developed, but is still very small, and is bounded by thick massive cells, there may be observed (Pl. 25, figs. 15 and 16) on each side in close apposition two cells which have the appearance in section of being pierced by an exceedingly fine, perfectly clean-cut canal. How these canals end I have not been able to determine: but subsequent stages show that these, with the cells that contain them, are the foundations of the excretory system.

At first these perforated cells are widely separated from the endocœle cavity and embedded in the thickness of the blastoderm. As the cavity increases in size their relative position becomes altered, until they come to lie directly below the lining membrane of the cavity. At first the entire structure consists on each side of two cells, a larger and a smaller, which have fused, and the substance of which has become perforated by a sinuous canal. This is destined to give rise to the terminal sac of the excretory system. The canal extends through several cells situated close to the first two, and in this way is formed the beginnings of the main longitudinal vessels. Later (Pl. 25, fig. 17), when the rudiment of the brain has become well advanced, the terminal sacs, while still retaining their position immediately under—i. e. external to—the membrane lining the cavity, and, while still continuing each to consist of only two fused cells perforated by a canal, assume a more complex structure, and take on, in all the most essential points, the structure which we have found to characterise them in the adult. The canal becomes wider in relation to the thickness of the enclosing wall; and from the main vessel is given off a slender branch—the future vesicular vessel—which, approaching the terminal sac on the inner side, breaks up into a number of excessively fine capillaries that ramify through the substance of the wall of the sac.

It will be seen from the above account of its mode of formation that the excretory system of Temnocephala is, in the strictest sense, of intracellular character. I am thus compelled to dissent from Goto's opinion (**4**, p. 71) that I was not justified in using that term, as well as to his more general view (p. 74) that "the term intracellular is quite inappropriate to the excretory system of the Trematodes and the Turbellaria."

Perhaps the most remarkable event in the history of the development of the excretory system in Temnocephala is the change which takes place in the position of the contractile terminal sacs. Originally, as we have seen, they are placed close to the epithelium of the endocœle, and apparently open into the latter. When the cavity becomes reduced, and the pharynx begins to become rounded off, the sacs lose their original connections, and, becoming displaced outwards, enter into connection with the epidermis, and open on the exterior on the dorsal surface. Thus, in a stage in which the rudiments of the tentacles are being formed, the sacs occupy the position which they retain in the adult.

VII. Development of the Alimentary System.

The alimentary system of Temnocephala consists of two principal parts—pharynx and intestine. The mouth, situated far forwards on the ventral surface, leads, through a very small cavity representing a pharyngeal sac, into the lumen of the pharynx. The latter is a large, rounded organ with thick walls of highly complex structure. The cavity is lined internally by a layer, the nature of which is by no means clear. By Weber (**18**) it has been described as a continuation of the cuticle of the integument. Monticelli (**14**) refers to it as a syncytial epithelium. Wacke (**16**) refers to it as an epithelium, and gives excellent figures of its minute structure. It is a non-cellular layer, in which a degenerate nucleus may sometimes be detected, but only quite exceptionally. It is composed of granular material, the granules of which are arranged in rows or strings, most, at least in the Australasian species, having a vertical direction. Many of these strings are traceable in some series into the thickness of the wall of the pharynx. The internal cuticle, described and figured by both Monticelli and Wacke as bounding this layer internally, is not a separate and distinct layer, but is merely the innermost stratum of the granular layer, and in most preparations is not differentiated at all.

Externally a limiting membrane forms a pharyngeal capsule, separating the muscular mass of the pharynx from the surrounding parenchyma. Between the external capsule and the internal epithelium, in addition to the elaborate system of muscular fibres, there are a number of cells and a system of nerve-fibres. The cells are of several kinds, comprising bipolar nerve-cells, excretory cells, and unicellular glands, the ducts of which are usually said to open into the cavity of the pharynx.

Posteriorly the pharynx leads through a short passage—the œsophagus—into the spacious intestine. Round the œsophagus are a number of unicellular "salivary" glands.

The intestine in all the Australasian species (and also in T. chilensis according to Wacke) is constricted at intervals by a number of annular muscular dissepiments, the number of these varying with the species. The epithelium is composed of long narrow cells, the majority of which, though probably mainly absorptive in function, contain vacuoles enclosing granules which are probably excretional, while others are of the nature of unicellular digestive glands.

The pharyngeal sac, the pharynx with the œsophagus, and the intestine are all derived from different sources. The first may be said to be of the nature of a stomodæum. The pharynx is derived from a portion of the wall of the endocœle. A number of large cells become arranged in a manner presenting the appearance of an epithelium on the wall of this region, separated from the internal space by the thin epithelial lining of the cavity. This specially modified portion of the wall then becomes rounded off as the wall of the pharynx, what remains of the cavity, which becomes much diminished in size, forming a short passage corresponding in position with the future œsophagus, and terminating abruptly behind in the mass of yolk.

The large cells in the wall of the embryonic pharynx at first form its entire thickness, with the exception of the exceedingly thin internal epithelium. But afterwards muscular fibres are developed both internally and externally. The mode of formation of these is not clear, but since no other elements come into play, there can be little doubt that a portion of the cells of the wall of the pharynx are of the nature of myoblasts. The rest become the nerve-elements and the glandular and excretory cells.

The mode of formation of the so-called epithelium of the pharynx is a matter of some interest. It is represented at first, as already pointed out, by the thin syncytial lining of that part of the endocœle from which the pharynx becomes developed; and at no subsequent stage does its epithelial character become more pronounced. On the contrary, as development advances, the cellular character of this layer becomes almost or completely lost. In fact, were it not for the occasional occurrence in it of a more or less altered nucleus, it might be supposed to be entirely non-cellular. But, though it loses, or nearly loses, its cellular character, this layer greatly increases in thickness, and, in the adult, maintains its thickness in spite of the loss of material to which it must be subjected as a result of constant wear and tear during the capture and ingestion of active living prey.

Up to a late stage in Temnocephala fasciata the rest of the digestive system is merely represented by a short passage, which continues back the lumen of the pharynx, and ends abruptly within the mass of yolk. This is the future œsophagus, and in its neighbourhood are a number of cells destined to become the unicellular or "salivary" glands. Thus, at a stage when the muscular wall of the pharynx, with its anterior and posterior sphincters, has reached an advanced stage of development, the intestine is not yet definitely represented.

At this stage the cavity of the pharynx is still completely shut off from the exterior by the septum formed, as already described, from the persistent thin roof of the original endocœle.

The stages by which the intestine, with its definite epithelium and septa, becomes differentiated, have not yet been traced. It seems probable, however, that a structure which makes its appearance below the endocœle, after the brain and excretory sacs have become formed, may be concerned in the formation of the primitive endoderm. At this stage (Pl. 25, fig. 20), a cleft or infolding appears among the cells on the floor of the cavity. Later this takes the form of a group of cells arranged around a small lumen—the appearance being very similar to that presented by the first beginnings of the endocœle itself. Since this is behind the brain and excretory sacs, and is too far forward to be of the nature of a genital primordium, it is permissible to suppose that it represents the earliest rudiment of the endodermal system. Its further history has not been traced; in fact, it soon disappears as such; but if the above supposition as to its nature be correct the cells to which it gives rise must extend round the yolk between it and the rest of the developing parts, and become converted into the intestinal epithelium.

In the latest stage observed within the egg—a stage with fully-developed eyes and abundant pigment, and with the male part of the reproductive apparatus far advanced—the intestine is still entirely devoid of lumen, and consists of a solid granular mass with numerous nuclei, which are most abundant in the peripheral zone. Slender strands passing inwards into this solid mass represent the future dissepiments. In the case of T. novæ-zealandiæ, however, in the latest stages from the egg the intestine has developed a lumen, and the epithelium is recognisable, though the septum still persists shutting off the cavity of the pharynx from the exterior. It is a remarkable fact, which is probably of significance in connection with the function of this persistent septum, that in nearly all sections of late stages with fully-developed pharynx the lumen of the latter is found to be filled with a mass of yolk-granules which have evidently become detached from the central body of yolk, and have been prevented by the occluding septum from reaching the exterior, i. e. the space between the embryo and the shell.

VIII. Development of the Epidermis.

In the integumentary system of Temnocephala the most characteristic feature is the presence of a well-developed nucleated epidermal layer of a syncytial character. In the embryo this is not represented by any definite primordium, such as has been observed to be formed in Polyclads (Lang and others) and Rhabdocœles (Caullery and Mesnil [**4**], Breslau [**3**]), and there is no process corresponding to the process of overgrowing of the embryo by an epidermal layer, such as occurs in these groups. When an epidermis is first discernible, it consists of a very thin membrane with wide-apart flattened nuclei, covering only the ventral surface, and there is no evidence of any process of proliferation such as must accompany the spreading of the edge of this layer by cell-division. It would appear, in fact, as if the epidermis were formed by cells migrating to the surface and there becoming flattened out and united together to form the syncytium.

Temnocephala fasciata, unlike T. minor and T. Dendyi, has no cilia on the surface in the adult, and I have found no trace of them at any stage in the development.

IX. Development of the Reproductive System.

The details of this process have not yet been followed out. In all the species examined the male part of the reproductive apparatus is developed at an earlier stage than the female. In the later stages within the egg the penis, the vesicula seminalis, the vasa deferentia, and the testes are all well advanced. In T. fasciata each testis is represented by a mass of primordial cells surrounded by indifferent cells. In T. novæ-zealandiæ before the young animal leaves the egg the first stages of spermatogenesis have occurred. At this stage the atrium does not yet open on the exterior. The definite development of the female part of the apparatus does not begin till after hatching, so that Wacke's statement that Temnocephala is protandrous appears to have some evidence in its favour, though a study of the post-larval development will be necessary in order to decide whether the condition is one of actual functional protandry, and not merely one of more active development of the male apparatus in the early stages.

The unique features of the early development of Temnocephala are associated with the formation of the remarkable internal cavity (endocœle) around which the foundations of various systems of organs are laid down. As nothing parallel to this cavity has, so far as my knowledge extends, been met with in other groups,[1] it is natural to inquire if its presence can be associated with any special conditions under which the development takes place—if its occurrence can be supposed to be of the nature of an adaptation.

The Australian fresh-water Crayfishes, on which live all the known Australian members of the Temnocephaleæ, shelter or support a great variety of small Invertebrata. A considerable proportion of these live in the branchial cavities, but many adhere to various parts of the outer surface. Among such dependents of the Crayfishes are Protozoans, Nematodes, Rhabdocœles, Rotifers, Stratiodrilus, Phreodrilus, a parasitic Hydrachnid, and others. In a country subject at times, as Australia is in many parts, to long-continued droughts, aquatic organisms must often suffer wholesale destruction as a result of the drying-up of the smaller streams. Crayfishes are able to avoid such a fate by sheltering between boulders or burrowing deeply in the bed of the stream. The animals adhering to them thus have a chance of survival denied to their free-living relatives. Yet it may, and doubtless does, often happen that even the Crayfishes are unable to escape the risk of desiccation. Under such circumstances the presence of a relatively large space filled with water in the interior of the egg of the Temnocephaleæ may make all the difference in enabling the embryo to retain its vitality until the dry period passes and the stream fills again.

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[1] It might be possible to trace some connection between the embryonal pharynx of the Tricladida (Hallez [**6**], Metschnikoff [**12**], Iijima [**10**]) and the endocœle of Temnocephala. Both cavities arise as spaces in rounded groups of cells in the blastoderm; but the former opens on the surface and swallows yolk cells, with which the intestine becomes distended. Moreover it is purely provisional, and disappears entirely, a new pharynx becoming developed in its place.

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[1] I have not been able to see this paper. What I have learnt regarding its contents has been obtained from Graff (5).

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EXPLANATION OF PLATES 23—25,[1]

Illustrating Professor W. A. Haswell's paper on "The Development of the Temnocephaleæ."

LETTERING.

br. Brain. *e.* Supposed endoderm primordium. *en.* Endocœle. *ep.* Epidermis. *ep. en.* Epithelial lining of endocœle. *ex.* Excretory sac or excretory cells. *ey.* Eye. *o.* Ovum. *o. p.* Oral papillæ. *ph.* Pharynx. *s.* Roof of endocœle, becoming septum, cutting off lumen of pharynx from the exterior. *t.* Tentacle. *y.* Yolk-cells. *y'.* Yolk syncytium.

PLATE 23.

FIG. 1.—Longitudinal section of the ovary of Temnocephala comes. × 500.

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[1] All the figures have been re-drawn by Mr. A. C. Cronin, of the Water and Sewerage Department, Sydney, from my original drawings.

Fig. 2.—Transverse section through uterine ovum of T. fasciata, passing through the unsegmented ovum. × 250.

Fig. 3.—Section through an early blastoderm in which three kinds of cells have become distinguishable. × 500.

PLATE 24.

Fig. 4.—Section through a later stage, with groups of larger, lighter, surrounded by smaller, more darkly stained, cells. × 500.

Fig. 5.—Section through a blastoderm in which the first definite trace of the cavity has made its appearance. × 500.

Fig. 6.—Later stage in the development of the cavity. × 500.

Fig. 7. Longitudinal section through the entire egg, with a more advanced cavity. × 170.

Fig. 8.—Portion of a longitudinal section of egg passing through the cavity, and cutting the blastoderm nearly transversely. × about 330.

Fig. 9.—Similar section of a later stage with the brain more advanced. × about 330.

Fig. 10.—Similar section, cutting the blastoderm nearly transversely, but with some obliquity, so that it passes through the brain and one excretory sac. × 330.

Fig. 11.—Anterior part of a vertical longitudinal section, approximately median, at a stage when the rudiments of the tentacles and eyes are appearing. × about 330.

PLATE 25.

Fig. 12.—Entire longitudinal and vertical section of embryo with the tentacles further developed than at the stage represented in fig. 11, and flexed backwards over the mouth. × about 130.

Fig. 13.—Anterior part of a longitudinal and approximately median and vertical section of an embryo with well-developed, ventrally flexed tentacles.|

Fig. 14.—Transverse section through the blastoderm in the region behind the endocœle at a stage somewhat later than that represented in fig. 10, showing the posterior prolongation comprising the genital rudiment. × about 500.

Figs. 15 and 16.—Successive sections of an embryo at an early stage in the formation of the endocœle, showing an early phase in the history of the cells destined to form one of the excretory sacs. × about 500.

Fig. 17.—Portion of a section passing through the endocœle at a stage similar to that represented in fig. 9, showing the developing excretory sac, and its connection with the endocœle.

Figs. 18 and 19.—The excretory sac in the adult; fig. 19 shows a part of the system of capillaries in which are numerous flame-cells, given off from the vesicular vessel and ramifying through the wall of the sac. Reproduced from a 'Monograph of the Temnocephaleæ,' pl. x, figs. 11 and 12.

Fig. 20.—Portion of a section of an egg with far advanced endocœle and distinct brain and excretory sacs, showing what is supposed to be the first trace of an endodermal primordium.